UNITED STATES ENVIRONMENTAL PROTECTION AGENCY WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

September 13, 2016

MEMORANDUM

Subject: Efficacy Review for Markie,
EPA Reg. File No. 3573-RNN,
DP Barcode: D434425

From: Son Nguyen
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

Thru: Mark Perry, Team Leader
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

To: Eric Miederhoff/Karen Leavy
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: The Proctor & Gamble Company
5299 Spring Grove Avenue
Cincinnati, OH 45217

Formulation from the Label:

Active Ingredient	% by wt.
Didecyl Dimethyl Ammonium Chloride	0.33
OtherIngredients	99.67
Total	100.00%

I. BACKGROUND

The product, Markie (EPA Reg. File #3573-RNN) is a new product, designed as a ready to use product and to be used as a hard, non-porous surface broad-spectrum disinfectant with bactericidal, virucidal, and hard and soft surface mold and mildew fungistatic activity. The product may also be used as a soft and hard non-food contact surface sanitizer. The registrant is citing all efficacy data from another identical/substantially similar product of the same company, Vesta (EPA Reg. #3573-99), and no new uses are to be added to the proposed label. The studies were conducted at Accuratus Lab Services, located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121.

This data package contained a letter from the applicant to EPA (dated June 10, 2016), EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-4 (Confidential Statement of Formula), EPA Form 8570-27 (Formulator's Exemption Statement), EPA Form 8570-4 (Confidential Statement of Formula (Basic and 11 Alternative Formulas), EPA Form 8570-27 (Formulator's Exemption), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), 14 cited efficacy studies (MRID Nos. 49081714 through 49081727) previously submitted for EPA Reg. #3573-99 (Vesta), and the product's proposed label. Statement of No Data Confidentiality Claims, Good Laboratory Practice Statement, and Quality Assurance Unit Summary were included with each study.

II. USE DIRECTIONS

The ready to use spray product is designed to be used to disinfect on hard, non-porous surfaces with bactericidal and virucidal activities, and with mold/mildew prevention activity (fungistatic) on hard and soft surfaces in household environment (broad-spectrum). The product is also intended to be used as sanitizer on both hard, non-porous surfaces and soft surfaces. The soft surfaces that the product is intended to be used on includes athletic apparel/gear, backpacks/school packs, bed sheets, bed pillow cases, car seats, comforters, couch, curtains, diaper bag, dog (pet) beds, draperies, duvet, (fabric) (window) treatments, (fabric) (laundry) bags, (fabric) gym bags, (fabric) shower curtains, high chair seat, kitchen seat (cushion), mattresses, mops (brooms), bath mats, shoes, slippers (house shoes), sofas (love seats), sports padding, stroller seat, (throw) pillows, towels (wash clothes) upholstery, (upholstered) chairs, (upholstered) couches, and (upholstered) furniture. The hard, non-porous surfaces that the product is intended to be used on includes bathroom surfaces, brass, cat litter boxes, changing tables, chrome, clean up carts, copper, counter (tables), crystal, cuspidors, desk, diaper pails, dish racks, door knob (handle), drains, dressing carts (tables) (racks), drinking fountains, enamel, faucets, fixtures, floors, garbage (cans) (pails) (bins) (lids), glass, glass topped furniture, glazed ceramic tile, glazed porcelain, (household) tools, laminate (surfaces), lamps, light switches, linen closets, linoleum, litter boxes, marble (cultured), marble (synthetic), marlite, metal, metal blinds, microwave (ovens) exteriors, mirrors, no wax floors, non-wood (outdoor) (patio) furniture, non-wood baby furniture, (non-wood) (linen) cabinets, non-wood chairs, parquet, pens, plastic laundry basket (hamper), plastics, porcelain (tile), recycling bins, refrigerator exteriors, salad bar sneeze guards, sealed granite, (shopping) (linen) carts, shower (curtain) (plastic) (liner), shower (stall) (area) (door) (rails), sinks (basin), sports equipment, stainless steel, tables (tabletops), telephones (cellular phones) (mobile phones), toilet (seats) (areas), hard non-porous areas under sink, urinal (exteriors), vinyl (tile), wheelchairs, whirlpool (Jacuzzi) (hot tub) interiors, and window/window sills.

Directions on the proposed label provide the following information regarding preparation and use of the product:

To sanitize soft surfaces (fabrics), clean heavily soiled areas before application. From a distance of 6-8 inches, spray a spot (2" x 2" area) evenly until damp to kill bacteria. Fabric must remain wet for 5 minutes.

For mold/mildew prevention (control) (inhibition): For heavily soiled areas, a pre-cleaning step is required. From a distance of 6-8 inches, spray surface until thoroughly wet. Fabric must remain wet for 5 minutes. Repeat treatment every 14 days, or more often if new growth appears.

For Sanitization and [Bactericidal & Virucidal†] Disinfection: For heavily soiled areas only, a pre-cleaning step is required. For hard non-porous surfaces, spray surface from 6-8 inches until thoroughly wet. Let stand for 5 minutes before wiping.

For mold/mildew prevention (control) (inhibition): For heavily soiled areas, a pre-cleaning step is required. For hard non-porous surfaces, spray surface from 6-8 inches until thoroughly wet. Let stand for 5 minutes before wiping. Repeat treatment every 7 days, or more often if new growth appears.

Not for use on food contact surfaces including dishes, glassware, cookware and utensils. A rinse is required for surfaces in direct contact with food.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces (Against a Broad Spectrum of Bacteria):
The effectiveness of disinfectants for use on hard surfaces must be substantiated by data derived using the AOAC Use-Dilution Method (UDM) (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products). Sixty carriers must be tested against each of the three batches of the product at the lower concentration limit (LCL). For UDM, the mean log density for *S. enterica* (ATCC 10708) is to be at least 5.0 and not above 6.0. A mean log density of at <5.0 or >6.0 invalidates the test. The mean log density for *S. aureus* (ATCC 6538) is to be at least 6 and not above 7.0. A mean log density <6.0 or >7.0 invalidates the test. For AOAC Germicidal Spray Products as Disinfectant Method, the mean log density for *S. enterica* (ATCC 10708) is to be at least 4.0 and not above 5.0. A mean log density of <4.0 or >5.5 invalidates the test. The mean log density for *S. aureus* (ATCC 6538) is to be at least 5.0 and not above 6.5. A mean log density <5.0 or >6.5 invalidates the test. To support products labeled as "general disinfectants", killing on 59 out of 60 carriers is required for germicidal spray testing is required. For UDM, conduct three independent tests (i.e., three batches at the LCL tested on three different test days) against the test microbe. The performance standard for *S. aureus* (ATCC 6538) is 0-3 positive carriers out of sixty. The performance standard for *S. enterica* (ATCC 10708) is 0-1 positive carriers out of sixty. Contamination of only one carrier (culture tube) is allowed per 60-carrier set; occurrence of more than one contaminated carrier invalidates the test results for both UDM and Germicidal Spray Products as Disinfectant Method. To be deemed an effective product, the product must pass all tests for both microbes. All products should meet the performance standard associated with the method and microbe at ≤ 10 minutes of contact. The above Agency standards are presented in OCSPP 810.2200.

Disinfectants for Use on Hard Surface Environments (Additional Microorganisms):
Effectiveness of disinfectants against specific bacteria other than those named in the designated test microorganism(s) is permitted, provided that the target microbe is likely to be present in or on the recommended use areas and surfaces. This section addresses efficacy testing for limited, broad-spectrum or hospital disinfectants which bear label claims against bacteria other than *S. enterica* (ATCC10708), *S. aureus* (ATCC 6538) or *P. aeruginosa* (ATCC 15442). The effectiveness of disinfectant against specific bacteria must be determined by AOAC Use-Dilution Method (UDM). Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. The product should kill all the test microorganisms on all carriers in ≤ten minutes. The minimum carrier count to make the test valid should be 1×10^4 CFU/carrier. For a valid test, no contamination of any carrier is allowed.

Virucides:
The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface

for each of 2 different product lots of disinfectant at LCL must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Sanitizer Test (for inanimate, non-food contact surfaces):

The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface over those on an untreated control surface. The Agency recommends the American Society for Testing and Materials (ASTM) Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (E1153) (Ref. 1). The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. For hard, porous surface label claims use unglazed ceramic tile. For hard, nonporous surface label claims use stainless steel carrier or glass slide. Use 5 test carriers and 3 control carriers. Tests should be performed with each of 3 product samples, representing 3 different product lots, tested at LCL against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). The ASTM method states that the inoculum employed should provide a count of at least 7.5×10^5 colony forming units per carrier. The performance measure should demonstrate a reduction of ≥99.9% (a 3-log10 reduction) in the number of each test microorganism over the parallel control count within 5 minutes.

Spot Soft Surface Sanitization:

The study is designed to evaluate the antimicrobial efficacy of sanitizers on pre-cleaned or lightly soiled, non-food contact soft surfaces. For sanitizer products intended for use on soft, non-food contact surfaces, a fabric carrier method is used to generate efficacy data. The test system proposed is a modification of the ASTM approved method for the evaluation of the antimicrobial efficacy of sanitizers on non-food contact surfaces. The method is modified for spray product application. The Agency recommends the use of The American Society for Testing and Materials (ASTM) Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (ASTM E1153-03). Three product samples, representing three different batches, at the LCL should be tested against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048). The ASTM method states "an average of at least 7.5×10^5 organisms must have survived the inoculated control squares for the test to be valid." Two different fabric types should be tested. The fabrics should represent natural fabrics, such as cotton, and synthetic fabrics, such as polyester or rayon. A film of bacterial cells, dried on fabric carriers, is exposed to the test substance for a specified contact time. After exposure, the carriers are transferred to vessel containing neutralizing subculture media and assayed for survivors. Appropriate viability and sterility of organism population and neutralization controls are performed. Carrier type claimed on the label must be consistent with the test system. The test material meets effectiveness requirements of kill an average of at least 99.9% (3 log reduction) of the required organism on the 5 replicate carriers within 5 minutes. Controls must always meet the stipulated criteria.

Hard Surface Mildew Fungistatic Test:

This method is intended to be used in supporting fungistatic claims for the control, treatment, or prevention of fungi and subsequent mildew growth on hard surfaces. Use of this test method in no way supports claims for use of a product as a fungicide. The test is to be conducted on 10 glazed ceramic tiles for each of two product lots against *Aspergillus niger* (ATCC 6275). Ten untreated glazed tiles are to be used as the control, on which greater than 50% of each tile is to be covered with fungal growth after 7 days for the test to be considered valid. Growth observations are to be made visually after 7 days of incubation. If no visible growth is evident at the end of the test period, examination at a 15X magnification must take place. A product dosage is considered acceptable when all ten treated replicates are free of fungal growth.

Fabric Mildew Fungistatic Test Method: The test is to be conducted on cotton muslin strips cut 25 by 75 mm from 136 to 203 g/m^2 (4 to 6 oz./yd.^{2}) fabric. The strips should be autoclaved sterilized. The product is to be tested against *Aspergillus niger* (ATCC 6275) and *Penicillium variable* (ATCC 32333). Soak fabric strips in Nutrient broth for three minutes or until saturated. Remove excess liquid and allow fabric strips to dry before proceeding with application of the test product. Both sides of ten strips for each batch should be spray treated with product. The application specifications including spray distance from nozzle, degree of wetness, draining conditions, and drying procedures should be reported. Equal volumes of well-agitated conidial suspensions of *Aspergillus niger* and *Penicillium variable* using a DeVilbiss atomizer (or equivalent) should be sprayed on both sides of each fabric strip. The fabric samples are suspended in individual 500 mL jars containing 90 mL water and incubated at approximately 28ºC with the caps tightened and backed off 1/8 turn to allow for ventilation. Observations are made weekly for four weeks or until treatments fail and abundant growth occurs on all treated strips (at 7, 14, or 21 days). Where no growth is visually evident at the end of the test period, examination at approximately 15X magnification must be conducted to confirm the absence or establish the presence of subvisual growth. The untreated control strips (10 strips) must have a minimum of 50% of their surface area covered with fungal growth after 7 days to consider the test valid. The acceptance criterion requires that all ten treated replicates per batch must be free of fungal growth. The directions for use must specify retreatment every 7, 14, or 21 days, as necessary depending on the length of time that all of the test strips remain free of mildew growth. Labeling of products which do not permit growth after four weeks incubation must specify a retreatment schedule, such as "repeat as necessary when new growth appears", and should indicate that treatments should be effective for at least 28 days.

Supplemental Claims:
An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum. On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy in hard water, all microorganisms (i.e., bacteria, fungi, and viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same tolerance level.

IV. SYNOPSIS OF SUBMITTED EFFICACY STUDY

According to the Certificate of Analyses dated May and July of 2012, the concentration of **Batch No. SS1684** was reported to be 0.2977% Didecyl Dimethyl Ammonium Chloride**,** of **Batch No. SS1685** was reported to be 0.2956% Didecyl Dimethyl Ammonium Chloride, and of **Batch No. SS1691 (aged)** was reported to be 0.3008% Didecyl Dimethyl Ammonium Chloride in April, 2012 and 0.2985% Didecyl Dimethyl Ammonium Chloride in July, 2012. The product's Didecyl Dimethyl Ammonium Chloride nominal concentration is 0.33% and the Lower Certified Limit is 0.297%.

1. **MRID 49081714 "AOAC Germicidal Spray Method," Test Organisms: *Salmonella enterica* (ATCC 10708), and *Staphylococcus aureus* (ATCC 6538) for Markie, EPA Reg. No. 3573-RNN, by Joshua Luedtke, M.S. Study conducted at Accuratus Lab Services. Study completion date – July 6, 2012. Project Identification No. A13414.**

This study was conducted against *Salmonella enterica* (ATCC 10708) and *Staphylococcus aureus* (ATCC 6538). Two batches (Batch# SS1684 and Batch# SS1685) of the product, Markie, were tested using Accuratus Lab Services Protocol No. PG30050312.GS.1 (copy provided). The test substance was received ready to use. An initial broth suspension was prepared by inoculating 10mL of culture broth from the stock slant. From the initial broth suspension, a minimum of three daily transfers using 1 loopful (10µL) of culture into 10mL of culture media were performed on consecutive days prior to use in the testing procedure. For both test organisms, nutrient broth growth medium was subcultured using a daily transfer (more than 3, but less than 30 transfers) of each test organism. For the final subcultured step, a sufficient number of 25 x 150

mm tubes containing 20mL of culture media were inoculated with 1 loopful (10µL) of culture. A 48- 54 hour broth culture incubated at 35-37°C was prepared. Each test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris. The removed portion was transferred to a sterile vessel and was used in testing. The culture included a 5% FBS organic soil load. Sixty (60) glass slides per product lot were inoculated with 10.0 µL of culture using a calibrated pipettor. The inoculum was uniformly spread over the entire surface of the slide contained in the Petri dish. The dish was covered immediately and the procedure repeated until all slides were individually inoculated. For *Staphylococcus aureus*, the slides were allowed to dry for 38 minutes at 35-37°C at a 50% relative humidity. For *Salmonella enterica*, the slides were allowed to dry for 30 minutes at 35-37°C at a 50% relative humidity. For each lot of test substance, test carriers were sprayed, in a horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches using 2 sprays. Test sprayers were primed prior to use. Following the spray treatment, each treated carrier was held at 20°C and 55% relative humidity for 5 minutes. Each treated carrier was then transferred using sterile forceps at staggered intervals to 20mL aliquots of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of growth. Representative subcultures showing growth were subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism. Controls included those for neutralization confirmation, purity, viability, sterility, and carrier population. The reported average carrier population control in CFU/carrier for *Salmonella enterica* (ATCC 10708) was 5.79 $\log_{10}$ and for Staphylococcus aureus (ATCC 6538) was 5.74 $\log_{10}$.

Note

No protocol amendments were required for this study. No protocol deviations occurred during this study.

2. **MRID 49081715 "AOAC Germicidal Spray Method," Test Organisms: *Salmonella enterica* (ATCC 10708), and *Staphylococcus aureus* (ATCC 6538) for Markie, EPA Reg. No. 3573-RNN, by Anne Stemper, B.S. Study conducted at Accuratus Lab Services. Study completion date – August 22, 2012. Project Identification No. A13831.**

This study was conducted against *Salmonella enterica* (ATCC 10708) and *Staphylococcus aureus* (ATCC 6538). One batch (Batch# SS1691) of the product, Markie, were tested using Accuratus Lab Services Protocol No. PG30071212.GS (copy provided). The test substance was received ready to use. An initial broth suspension was prepared by inoculating 10mL of culture broth from the stock slant. From the initial broth suspension, a minimum of three daily transfers using 1 loopful (10µL) of culture into 10mL of culture media were performed on consecutive days prior to use in the testing procedure. For both test organisms, nutrient broth growth medium was subcultured using a daily transfer (more than 3, but less than 30 transfers) of each test organism. For the final subcultured step, a sufficient number of 25 x 150 mm tubes containing 20mL of culture media were inoculated with 1 loopful (10µL) of culture. A 48- 54 hour broth culture incubated at 35-37°C was prepared. Each test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris. The removed portion was transferred to a sterile vessel and was used in testing. The culture included a 5% FBS organic soil load. Sixty (60) glass slides per product lot were inoculated with 10.0 µL of culture using a calibrated pipettor. The inoculum was uniformly spread over the entire surface of the slide contained in the Petri dish. The dish was covered immediately and the procedure repeated until all slides were individually inoculated. For *Staphylococcus aureus*, the slides were allowed to dry for 39 minutes at 35-37°C at a 55.7% relative humidity. For *Salmonella enterica*, the slides were allowed to dry for 30 minutes at 35-37°C at a 55.7% relative humidity. For each lot of test substance, test carriers were sprayed, in a horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches using 2 sprays. Following the spray treatment, each treated carrier was held at 20°C and 58% relative humidity for 5 minutes. Each treated carrier was then transferred using sterile forceps at staggered intervals to 20mL aliquots of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80. All subcultures were incubated for 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for two days prior to examination. Following incubation,

the subcultures were visually examined for the presence or absence of growth. Controls included those for neutralization confirmation, purity, viability, sterility, and carrier population. The reported average carrier population control in CFU/carrier for *Salmonella enterica* (ATCC 10708) was 6.26 $\log_{10}$ and for Staphylococcus aureus (ATCC 6538) was 5.91 $\log_{10}$.

3. **MRID 49081716 "AOAC Germicidal Spray Method," Test Organisms: *Proteus mirabilis* (ATCC 7002) for Markie, EPA Reg. No. 3573-RNN, by Nicole Albert, B.S. Study conducted at Accuratus Lab Services. Study completion date – September 26, 2012. Project Identification No. A13383.**

This study was conducted against *Proteus mirabilis* (ATCC 7002). Two batches (Batch# SS1684 and Batch# SS1685) of the product, Markie, were tested using Accuratus Lab Services Protocol No. PG30050312.GS.2 (copy provided). The test substance was received ready to use. An initial broth suspension was prepared by inoculating 10mL of culture broth from the stock slant. From the initial broth suspension, a minimum of three daily transfers using 1 loopful (10µL) of culture into 10mL of culture media were performed on consecutive days prior to use in the testing procedure. Nutrient broth growth medium was subcultured using a daily transfer (more than 3, but less than 30 transfers) of each test organism. For the final subcultured step, a sufficient number of 25 x 150 mm tubes containing 20mL of culture media were inoculated with 1 loopful (10µL) of culture. A 48- 54 hour broth culture incubated at 35-37°C was prepared. Each test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris. The removed portion was transferred to a sterile vessel and was used in testing. The culture included a 5% FBS organic soil load. Ten (10) glass slides per product lot were inoculated with 10.0 µL of culture using a calibrated pipettor. The inoculum was uniformly spread over the entire surface of the slide contained in the Petri dish. The dish was covered immediately and the procedure repeated until all slides were individually inoculated. The slides were allowed to dry for 32 minutes at 25-30°C and at a 66% relative humidity. Each test substance sprayer was primed prior to use. For each lot of test substance, test carriers were sprayed, in a horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches using 2 sprays. Following the spray treatment, each treated carrier was held at 21°C and 53% relative humidity for 5 minutes. Each treated carrier was then transferred using sterile forceps at staggered intervals to 20mL aliquots of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80. All subcultures were incubated for 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for 2 days prior to examination. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for neutralization confirmation, purity, viability, sterility, and carrier population. The reported average carrier population control in CFU/carrier for *Proteus mirabilis* (ATCC 7002) was 6.34 $\log_{10}$.

Note
No protocol amendments were required for this study. No protocol deviations occurred during this study.

4. **MRID 49081717 "AOAC Germicidal Spray Method," Test Organisms: *Escherichia coli* (ATCC 11229) for Markie, EPA Reg. No. 3573-RNN, by Joshua luedtke, M.S. Study conducted at Accuratus Lab Services. Study completion date – September 6, 2012. Project Identification No. A13384.**

This study was conducted against *Escherichia coli* (ATCC 11229). Two batches (Batch# SS1684 and Batch# SS1685) of the product, Markie, were tested using Accuratus Lab Services Protocol No. PG30050312.GS.3 (copy provided). The test substance was received ready to use. An initial broth suspension was prepared by inoculating 10mL of culture broth from the stock slant. From the initial broth suspension, a minimum of three daily transfers using 1 loopful (10µL) of culture into 10mL of culture media were performed on consecutive days prior to use in the testing procedure. For both test organisms, nutrient broth growth medium was subcultured using a daily transfer (more than 3, but less than 30 transfers) of each test organism. For the final subcultured step, a sufficient number of 25 x 150 mm tubes containing 20mL of culture media were

inoculated with 1 loopful (10µL) of culture. A 48- 54 hour broth culture incubated at 35-37°C was prepared. Each test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris. The removed portion was transferred to a sterile vessel and was used in testing. The culture included a 5% FBS organic soil load. Ten (10) glass slides per product lot were inoculated with 10.0 µL of culture using a calibrated pipettor. The inoculum was uniformly spread over the entire surface of the slide contained in the Petri dish. The dish was covered immediately and the procedure repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at a 50% relative humidity. For each lot of test substance, test carriers were sprayed, in a horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches using 2 sprays. Test sprayers were primed prior to use. Following the spray treatment, each treated carrier was held at 21°C and 56% relative humidity for 5 minutes. Each treated carrier was then transferred using sterile forceps at staggered intervals to 20mL aliquots of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80. All subcultures were incubated for 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for 2 days prior to examination. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for neutralization confirmation, purity, viability, sterility, and carrier population. The reported average carrier population control in CFU/carrier for *Escherichia coli* (ATCC 11229) was 6.74 $\log_{10}$.

5. **MRID 49081718 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Test Organisms: Influenza A virus for Markie, EPA Reg. No. 3573-RNN, by Dawn Pierson, B.S. Study conducted at Accuratus Lab Services. Study completion date – June 11, 2012. Project Identification No. A13314.**

This study was conducted against Influenza A virus, ATCC VR-544, Strain Hong Kong. The strain was obtained from the American Type Culture Collection, Manassas. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soul load. Cultures of Rhesus Monkey kidney (RMK) cells were obtained from ViroMed Laboratories, Inc., Cell Culture Division and were used as the indicator cell line in the infectivity assays. Two batches (Batch# SS1684 and SS1685) of the product, Markie, were tested using Accuratus Lab Services Protocol No. PG30051112.FLUA (copy provided). The test substance was ready to use as received from the Sponsor. It was equilibrated to the exposure temperature prior to use and was applied according to the use directions provided by the Sponsor. Films of virus were prepared by spreading 200µL of virus inoculum uniformly over the bottoms of fifteen separate 100 x 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus films were dried at 20.0°C in a relative humidity of 40% until visibly dry (20 minutes). For each batch of test substance, five dried virus films were individually exposed for 5 minutes at room temperature 20.0°C to the amount of spray released under use conditions. The carriers were sprayed until thoroughly wet 2 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. Prior to titration, the filtrates (10^{-1} dilution) were passed through a second Sephadex column, to aid in removing the cytotoxic effect of the test substance to the indicator cell cultures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 1% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Cells in multiwell culture dishes were inoculated in quadruplicate with 100µL of the dilutions prepared from test and control groups. The input virus control was inoculated in duplicate. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were scored periodically for seven days for the absence of presence of CPE, cytotoxicity, and for viability. Viral and cytotoxicity titers are expressed as $-\log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity (TCD_{50}), respectively, as calculated by the method of Spearman Karber. Controls included those for input virus control, dried virus control film, cytotoxicity, and

neutralization. The average dried virus control (in $TCID_{50}$/100µL) obtained for Influenza A virus, ATCC VR-544, Strain Hong Kong was 5.18 log_{10}.

Note
No protocol amendments were required for this study. No protocol deviations occurred during this study.

6. **MRID 49081719 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces", Test Organisms: Influenza A virus for Markie, EPA Reg. No. 3573-RNN, by Shanen Conway, B.S. Study conducted at Accuratus Lab Services. Study completion date – August 17, 2012. Project Identification No. A13817.**

This study was conducted against Influenza A virus, ATCC VR-544, Strain Hong Kong. The strain was obtained from the American Type Culture Collection, Manassas. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soul load. Cultures of Rhesus Monkey kidney (RMK) cells were obtained from ViroMed Laboratories, Inc., Cell Culture Division and were used as the indicator cell line in the infectivity assays. One batch (Batch# SS1691) of the product, Markie, was tested using Accuratus Lab Services Protocol No. PG30051112.FLUA (copy provided). The test substance was ready to use as received from the Sponsor. It was equilibrated to the exposure temperature prior to use and was applied according to the use directions provided by the Sponsor. Films of virus were prepared by spreading 200µL of virus inoculum uniformly over the bottoms of fifteen separate 100 x 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus films were dried at 20.0°C in a relative humidity of 40% until visibly dry (20 minutes). Five dried virus films were individually exposed for 5 minutes at room temperature 20.0°C to the amount of spray released under use conditions. The carriers were sprayed until thoroughly wet 2 sprays at a distance of 6-8 inches and held covered for the exposure time. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. To reduce the toxicity of the test substance to the indicator cell cultures, the 10^{-1} dilutions were passed through two additional individual previously prepared Sephadex columns prior to titration. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 1% (v/v) heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Cells in multiwell culture dishes were inoculated in quadruplicate with 100µL of the dilutions prepared from test and control groups. The input virus control was inoculated in duplicate. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were scored periodically for seven days for the absence of presence of CPE, cytotoxicity, and for viability. Viral and cytotoxicity titers are expressed as $-log_{10}$ of the 50 percent titration endpoint for infectivity ($TCID_{50}$) or cytotoxicity (TCD_{50}), respectively, as calculated by the method of Spearman Karber. Controls included those for input virus control, dried virus control film, cytotoxicity, and neutralization. The average dried virus control (in $TCID_{50}$/100µL) obtained for Influenza A virus, ATCC VR-544, Strain Hong Kong was 5.43 log_{10}.

7. **MRID 49081720 "EPA Hard Surface Mildew-Fungistatic Test", Test Organisms: *Aspergillus niger* (ATCC 6275), for Markie, EPA Reg. No. 3573-RNN, by Mathew Sathe, B.S. Study conducted at Accuratus Lab Services. Study completion date – June 21, 2012. Project Identification No. A13361.**

This study was conducted against *Aspergillus niger* (ATCC 6275). Two batches (Batch# SS1684 and SS1685) of the product, Markie, were tested using Accuratus Lab Services Protocol No. PG30050312.MSTAT (copy provided). The test substance was ready to use (RTU). The test substance was homogenous as determined by visual observation. The conidial suspension was prepared by inoculating a flask of Sabouraud Agar (Modified) with the stock culture and incubated for 10 days at 25-30°C. Following

incubation, sterile saline/Triton Solution (0.85% saline + 0.05% Triton X-100) and sterile glass beads were added to the flask. The flask was agitated to remove mycelia/conidia from the agar. The conidia suspension was aspirated from the flask and passed through sterile gauze to remove hyphal fragments. The suspension was added to a sterile tissue grinder and macerated. The conidial concentration was estimated by counting in a hemacytometer. The viable cell count was 1.4 x 10^8 conidia/mL. The conidial suspension was standardized to contain an approximate target of 1 x 10^7 conidia per mL by combining 1.00mL of unadjusted culture to 19.0mL of sterile 0.85% Saline + 0.05% Triton X-100. A 1.0mL aliquot of this suspension was added to 20.0mL of sterile Czapek's solution. A 1.00mL aliquot of FBS was added to 19.0mL of prepared Czapek/organism suspension to yield a 5% Fetal Bovine Serum organic soil load. One inch by one inch glazed ceramic tiles were sterilized for ≥2 hours at ≥180°C in a hot air oven and were used as carriers. For each lot of the prepared test substance, the surfaces of 10 carriers were sprayed with the test substance at a distance of 6-8 inches using 2 sprays. The test sprayers were primed prior to use. The treated carriers were placed in a vertical or near vertical position to permit excess liquid to drain. The carriers were dried in Petri dishes at 35-37°C for 41 minutes with the lids ajar. Untreated carriers were placed in sterile Petri dishes (10 carriers total) and dried for 41 minutes at 35-37°C with the lids ajar alongside the test carriers. Following the initial drying period, an atomizer was used to spray the surface of each test carrier and control carrier with the *Aspergillus niger* conidia-Czapek suspension. Approximately 5 sprays were used to apply the test organism. Carriers contained in Petri dishes were returned to a 35-37°C incubator and dried with the lids slightly ajar for 39 minutes until visibly dry. Each carrier (sprayed side up) was placed onto an individual water agar plate. All plates were incubated for 7 days at 25-30°C in a minimum of 95% relative humidity. The purity control was incubated for 44-76 hours at 25-30°C. All test and control carriers were examined after 7 days of incubation. The absence of fungal growth on all carriers is the criterion for determining the effectiveness of the test product. No visual growth was evident at the end of the 7 days on test carriers therefore a magnified examination was performed. To be considered a valid test, each untreated control carrier must be at least 50% covered with fungal growth after the 7 days. Controls included sterility, purity, and untreated control carriers.

Note
No protocol amendments were required for this study. No protocol deviations occurred during this study.

8. MRID 49081721 "Fabric Mildew-Fungistatic Test", Test Organisms: *Aspergillus niger* (ATCC 6275) and *Penicillium variabile* (ATCC 32333), for Markie, EPA Reg. No. 3573-RNN, by Joshua Luedtke, M.S. Study conducted at Accuratus Lab Services. Study completion date – August 29, 2012. Project Identification No. A13651.

This study was conducted against *Aspergillus niger* (ATCC 6275) and *Penicillium variabile* (ATCC 32333). Two batches (Batch# SS1684 and SS1685) of the product, Markie, were tested using Accuratus Lab Services Protocol No. PG30050312.FMSTAT (copy provided). The test substance was ready to use (RTU). The test substance was homogenous as determined by visual observation. The *Aspergillus niger* conidial suspension was prepared by inoculating a flask of Sabouraud Agar (Modified) with the stock culture and incubated for 10 days at 25-30°C. Following incubation, sterile saline/Triton Solution (0.85% saline + 0.05% Triton X-100) and sterile glass beads were added to the flask. The flask was agitated to remove mycelia/conidia from the agar. The conidia suspension was aspirated from the flask and passed through sterile gauze to remove hyphal fragments. The conidial concentration was estimated by counting in a hemacytometer. The conidial count was 1.3 x 10^8 conidia/mL. The conidial suspension was standardized to contain an approximate target of 5 x 10^6 conidia per mL by combining 1.00mL of unadjusted culture to 25.0mL of sterile 0.85% Saline. The *Penicillium variable* conidial suspension was prepared by inoculating 20 Potato Dextrose agar plates (see protocol deviation) and incubating at 25-30°C for 10 days. Following incubation, 2.0 mL of sterile saline/Triton Solution (0.85% Saline + 0.05% Triton X-100) was added to each plate harvested. The growth was harvested from the agar surface using a cell scrape. The harvested growth was transferred to a sterile vessel containing sterile beads and was shaken thoroughly. The culture was then filtered through sterile gauze to remove hyphal fragments. The conidial concentration was estimated by counting in a

hemacytometer. The conidial count was 9.15 x 10^8 conidia/mL. The conidial suspension was standardized to contain an approximate target of 5 x 10^6 condia per mL by combining 1.00 mL of unadjusted culture with 182.0 mL of 0.85% saline. Cotton muslin fabric carriers were cut as approximately 25mm by 75mm strips from 136 to 203g/m^2 (4 to 6 oz/yd^2) cotton muslin and were autoclave. Polyester fabric carriers were cut as approximately 25mm by 75mm strips and were autoclave. The sterilized fabric carriers were saturated with sterile glycerol nutrient solution by soaking the carriers for approximately three minutes until saturated. The excess liquid was squeezed out and the fabric carriers were allowed to dry prior to use. For each lot of the prepared test substance, each side of 10 test carriers were sprayed with the test substance at a distance of 6-8 inches using 2 sprays. The carriers were placed in a vertical or near vertical position to permit excess liquid to drain. The carriers were dried in Petri dishes at room temperature (22.1-22.4°C) for 3 hours until dry. Equal volumes (20.0mL) of each well-mixed conidial suspension were combined. A 2.00 mL aliquots of FBS was added to 38.0 mL of the combined organism suspension to yield a 5% Fetal Bovine Serum soil load. A 25.0mL aliquot of combined organism suspension/soil load was transferred to a Devilbiss atomizer. Both sides of each fabric test carrier strip was lightly sprayed using 10 sprays. The culture was mixed periodically within the atomizer during spraying. The fabric test and control samples were suspended in individual 250 mL French Square bottles containing approximately 10 mL sterile deionized water and incubated at 25-30°C. The caps were tightened and then backed off approximately 1/8 turn to allow for ventilation. It was ensured that no fabric was touching the water at the time of incubation. The control plates and organic soil load sterility control were incubated for 2 days at 25-30°C. Observations were made and recorded every 7 days for four weeks. The presence or absence of observable mold on the test carriers was the criterion for determining the effectiveness of the test product. Where no growth was visually evident at each weekly observation, a magnified examination was conducted to confirm the absence or establish the presence of sub-visual growth. The results of the test must be correlated to the intended label claim. The directions for use must specify treatment every 7 days, 14 days or 21 days, as necessary depending on the length of time that all test strips remained free of mildew growth. Labeling of products which do not permit growth after four weeks of incubation must specify a retreatment schedule such as "repeat necessary when new growth appears", and should indicate the treatments should be effective for at least 28 days. Controls included sterility, purity, initial suspension, and untreated control carrier. To be considered a valid control, each untreated control carrier must be covered with at least 50% of fungal growth over the total surface area after 7 days of incubation.

Note
No protocol amendments were required for this study.

Protocol Deviation: Inoculating of *Penicillium variabile* (ATCC 32333) for preparation of the conidial suspension was performed using Potato Dextrose Agar (PDA). The protocol indicates that the growth medium should have been Sabouraud Dextrose Agar; therefore this resulted in a protocol deviation. This deviation had no impact on the study as the organism growth on PDA was sufficient to achieve the required test organism concentration required for testing.

9. **MRID 49081722 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Spray Product Application)", Test Organisms: *Enterobacter aerogenes* (ATCC 13048) and *Staphylococcus aureus* (ATCC 6538), for Markie, EPA Reg. No. 3573-RNN, by Anne Stemper, B.S. Study conducted at Accuratus Lab Services. Study completion date – May 17, 2013. Project Identification No. A14923.**

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Three batches (Batch# SS1684, SS1685, and SS1691) of the product, Markie, were tested using Accuratus Lab Services Protocol No. PG30042513.NFS (copy provided). The test substance was received ready to use (RTU). The "initial broth suspension" was prepared by inoculating an initial tube (10mL) of culture broth with 5 transfers from the original stock. A minimum of three daily transfers using 1 loopful (10 µL) of culture into 10mL of culture media was performed on consecutive days prior to use as an inoculum. Each daily transfer was incubated for 24±2 hours using the appropriate growth medium (Tryptic

Soy Broth for *E. aerogenes*, and Nutrient Broth for *S. aureus*). A 48 hour culture was vortex-mixed and allowed to settle for ≥15 minutes. The upper 2/3rds of the culture was removed and transferred to a sterile vessel for use in testing. A 0.10mL aliquot of FBS was added to 1.90mL of each prepared culture to yield a 5% Fetal Bovine Serum organic soil load. Glass 1" x 1" carriers were inoculated with 0.02 mL (20.0 µL) of culture using a calibrated pipettor spreading the inoculum to within approximately 3mm of the edges of the carrier. The inoculated carrier were dried for 35 minutes at 35-37°C and 40% relative humidity with the Petri dish lids slightly ajar. A constant humidity chamber was used in place of a desiccating chamber to ensure uniform humidification conditions and to overcome slow re-equilibration of a desiccator after opening. The sprayer for each test substance was primed prior to use in testing. Following the completion of drying, each of the five test carriers were was sprayed using staggered intervals. Carriers were sprayed at a distance of 6-8 inches using 2 sprays and were allowed to expose at room temperature (21°C) and 37% relative humidity for 5 minutes. Following exposure, each carrier was transferred to 20 mL of neutralizer (Letheen Broth + 0.07% Lecithin + 0.5% Tween 80) using identical staggered intervals. Following the neutralization of the test carriers, the excess liquid in each Petri dish was transferred to the neutralizer jar containing the matching carrier. The jars were vortex-mixed to suspend the surviving organisms. For *E. aerogenes*, within 30 minutes of neutralization, duplicate 1.00mL and 0.100 mL aliquots of the neutralized solution (10°) were plated onto the recovery agar plate (Tryptic Soy Agar with 5% Sheep Blood (BAP)). For *S. aureus*, within 30 minutes of neutralization, duplicate 1.00mL and 0.100 mL aliquots of the neutralized solution were transferred to individual filter units pre-wetted with 10.0mL of sterile diluent. The contents were evacuated and each filter was rinsed with ≥50mL of sterile diluent. Each filter was transferred to the recovery agar medium using sterile forceps. The *S. aureus* plates were incubated at 35-37°C for 48 hours. The *E. aerogenes* plates were incubated at 25-30°C for 48 hours. Following incubation, the subcultures were visually enumerated. Controls included carrier population control, sterility, purity, neutralization confirmation, and inoculum count. The reported average carrier population control for *Staphylococcus aureus* (ATCC 6538) was 3.02×10^6 CFU/carrier, and for *Enterobacter aerogenes* (ATCC 13048) was 1.78×10^7 CFU/carrier.

Note

No protocol amendments were required for this study. No protocol deviations occurred during this study.

10. **MRID 49081723 "Standard Test Method for Efficacy of Sanitizers Recommended for Soft Non-Food Contact Surfaces (Modification for Spray Product Application)", Test Organisms: *Enterobacter aerogenes* (ATCC 13048) and *Staphylococcus aureus* (ATCC 6538), for Markie, EPA Reg. No. 3573-RNN, by Jill Ruhme, B.S. Study conducted at Accuratus Lab Services. Study completion date – September 5, 2012. Project Identification No. A13411.**

This study was conducted against *Enterobacter aerogenes* (ATCC 13048) and *Staphylococcus aureus* (ATCC 6538). Two batches (Batch# SS1684 and SS1685) of the product, Markie, were tested using Accuratus Lab Services Protocol No. PG30050312.NFS.1 (copy provided). The test substance was received ready to use (RTU) and was homogenous as determined by visual observation. The "initial broth suspension" was prepared by inoculating an initial tube (10mL) of culture broth with 5 transfers from the original stock. A minimum of three daily transfers using 1 loopful (10 µL) of the initial broth suspension into 10mL of culture media was performed on consecutive days prior to use. The appropriate growth medium (Tryptic Soy Broth for *E. aerogenes*, and Nutrient Broth for *S. aureus*) was subcultured using a daily transfer (more than 3, but less than 30 transfers) for each organism. A 48-54 hour culture was vortex-mixed and allowed to settle for ≥15 minutes. The upper 2/3rds of the culture was removed and transferred to a sterile vessel for use in testing. The cultures were thoroughly mixed prior to use. A 0.10mL aliquot of FBS was added to 1.90mL of each prepared culture to yield a 5% Fetal Bovine Serum organic soil load. Two types of carriers (cotton weave fabric and polyester fabric) were prepared. The cotton weave fabric contained 80 x 80 threads/inch plain cotton weave. A 1206.88 gram sample of test fabric was added to 12L of scouring solution containing 6.0331 grams of Na_2CO_3, 6.1295 grams of Triton X-100, and 12L of deionized water. The polyester fabric was 100 % Dacron polyester. An 80.20 gram sample of test fabric was added to 0.8L of scouring solution containing 0.4032 grams of Na_2CO_3, 0.4199 grams of Triton X-100, and 0.8L of deionized water. Each solution was allowed to boil for approximately 60 minutes. The fabric was removed and was

rinsed by first placing the fabric into boiling water for a minimum of 5 minutes and then placing the fabric into col water for a minimum of 5 minutes. During the rinsing procedure, the fabric was mixed in order to help remove the wetting agent. The fabric was allowed to air dry. The carriers were cut from the fabric to a size of approximately 1 inch x 1 inch and were autoclave sterilized. After sterilization, each carrier was placed into a sterile Petri dish prior to use in testing. Sterile carriers were inoculated with 0.02 mL (20.0 µL) of culture using a calibrated pipettor distributing the inoculum evenly about the carrier. The inoculated carriers were dried for 20 minutes at 35-37°C and 40% relative humidity with the Petri dish lids intact. After drying, each of the five test carriers, per carrier type, per test organism, was sprayed with the test substance using staggered intervals at a distance of 6-8 inches using 2 sprays and were allowed to expose at room temperature (20°C) and 43-62% relative humidity for 5 minutes. Following exposure, each carrier was transferred to 20 mL of neutralizer (Letheen Broth + 1.0% Lecithin + 9.0% Tween 80 [June 12, 2012] and Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 [July 6, 2012]) using identical staggered intervals. Following the neutralization of the test carriers, the excess liquid in each Petri dish was transferred to the neutralizer jar containing the matching carrier. The carriers were vortex-mixed. Glass beads were utilized to aid in organism recovery. For *E. aerogenes* testing performed June 12, 2012, within 30 minutes of neutralization, duplicate 1.00mL and 0.100 mL aliquots of the neutralized solution (10°) were plated onto the recovery plate medium (Tryptic Soy Agar with 5% Sheep Blood (BAP)). For *S. aureus* testing on cotton carriers on Lot SS1685 performed June 12, 2012, within 30 minutes of neutralization, duplicate 1.00mL aliquots of the neutralized solution (10°) and duplicate 1.00mL aliquots of a ten-fold serial dilution (10^{-1}) were plated onto the recovery plate medium. For *S. aureus* testing performed July 6, duplicate 1.00mL and 0.10 mL aliquots of the neutralized solution were transferred to individual filter units pre-wetted with 10.0mL of sterile diluent. The contents were evacuated and each filter was rinsed with ≥50mL of sterile diluent. Each filter was transferred to the recovery plate medium. The *S. aureus* plate were incubated at 35-37°C for 48±4 hours. The *E. aerogenes* plates were incubated for 48±4 hours at 25-30°C. For testing performed July 6, 2012, the *S. aureus* subcultes were placed at 2-8°C for 3 days prior to examination. Following incubation and storage, the subcultures were visually enumerated. Controls included carrier population control, sterility, purity, neutralization confirmation, and inoculum count.

Note
Testing performed on June 12, 2012, against *Staphylococcus aureus* did not demonstrate neutralization of SS1684 and SS1685 on polyester carriers and SS1684 on cotton carriers. Testing for SS1684 and SS1685 against *Staphylococcus aureus* was repeated using both cotton and polyester carriers on July 6, 2012, using filter neutralization. Valid data was generated and is contained in the body of this report.

No protocol amendments were required for this study.
Protocol Deviation: The protocol states that invalid results due to a test control failure may be repeated under the same control. In repeat testing on 7/6/12, SS1685 was inadvertently repeated on cotton carriers against *Staphylococcus aureus* even though the initial results were valid. This deviation did not impact the integrity of the study as both data sets were valid and therefore acceptable.

11. **MRID 49081724 "Standard Test Method for Efficacy of Sanitizers Recommended for Soft Non-Food Contact Surfaces (Modification for Spray Product Application)", Test Organisms: *Enterobacter aerogenes* (ATCC 13048) and *Staphylococcus aureus* (ATCC 6538), for Markie, EPA Reg. No. 3573-RNN, by Anne Stemper, B.S. Study conducted at Accuratus Lab Services. Study completion date – October 12, 2012. Project Identification No. A13830.**

This study was conducted against *Enterobacter aerogenes* (ATCC 13048) and *Staphylococcus aureus* (ATCC 6538). One batch (Batch# SS1691) of the product, Markie, was tested using Accuratus Lab Services Protocol No. PG30071212.NFS (copy provided). The test substance was received ready to use (RTU) and was homogenous as determined by visual observation. The "initial broth suspension" was prepared by inoculating an initial tube (10mL) of culture broth with 5 transfers from the original stock. A minimum of

three daily transfers using 1 loopful (10 µL) of the initial broth suspension into 10mL of culture media was performed on consecutive days prior to use. The appropriate growth medium (Tryptic Soy Broth for *E. aerogenes*, and Nutrient Broth for *S. aureus*) was subcultured using a daily transfer (more than 3, but less than 30 transfers) for each organism. A 48-54 hour culture was vortex-mixed and allowed to settle for ≥15 minutes. The upper 2/3rds of the culture was removed and transferred to a sterile vessel for use in testing. The cultures were thoroughly mixed prior to use. A 0.10mL aliquot of FBS was added to 1.90mL of each prepared culture to yield a 5% Fetal Bovine Serum organic soil load. Two types of carriers (cotton weave fabric and polyester fabric) were prepared. The cotton weave fabric contained 80 x 80 threads/inch plain cotton weave. A 568.88 gram sample of test fabric was added to 5.7L of scouring solution containing 2.8531 grams of Na_2CO_3, 2.8519 grams of Triton X-100, and 5.7L of deionized water. The polyester fabric was 100% Dacron polyester. A 417.78 gram sample of test fabric was added to 4.2L of scouring solution containing 2.1211 grams of Na_2CO_3, 2.1502 grams of Triton X-100, and 4.2L of deionized water. Each solution was allowed to boil for approximately 60 minutes. The fabric was removed and was rinsed by first placing the fabric into boiling water for a minimum of 5 minutes and then placing the fabric into col water for a minimum of 5 minutes. During the rinsing procedure, the fabric was mixed in order to help remove the wetting agent. The fabric was allowed to air dry. The carriers were cut from the fabric to a size of approximately 1 inch x 1 inch and were autoclave sterilized. After sterilization, each carrier was placed into a sterile Petri dish prior to use in testing. Sterile carriers were inoculated with 0.02 mL (20.0 µL) of culture using a calibrated pipettor distributing the inoculum evenly about the carrier. The inoculated carriers were dried for 20 minutes at 35-37°C and 40% relative humidity with the Petri dish lids intact. After drying, each of the five test carriers was sprayed with the test substance using staggered intervals at a distance of 6-8 inches using 2 sprays and were allowed to expose at room temperature (20°C) and 61% relative humidity for 5 minutes. Following exposure, each carrier was transferred to 20 mL of neutralizer (Letheen Broth + 0.28% Lecithin + 2.0% Tween 80 [August 9, 2012] and Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 [September 4, 2012]) using identical staggered intervals. Following the neutralization of the test carriers, the excess liquid in each Petri dish was transferred to the neutralizer jar containing the matching carrier. The carriers were vortex-mixed. For testing performed August 9, 2012 for *E. aerogenes*, within 30 minutes of neutralization, duplicate 1.00mL and 0.100 mL aliquots of the neutralized solution (10°) were plated onto the recovery plate medium (Tryptic Soy Agar with 5% Sheep Blood (BAP)). For testing performed September 4, 2012 for *S. aureus*, within 30 minutes of neutralization, duplicate 1.00mL and 0.10mL aliquots of the neutralized solution were transferred to individual filter units pre-wetted with 10.0mL of sterile diluent. The contents were evacuated and each filter was rinsed with ≥50mL of sterile diluent. Each filter was transferred to the recovery plate medium. The *S. aureus* plate were incubated at 35-37°C for 48±4 hours. The *E. aerogenes* plates were incubated for 48±4 hours at 25-30°C. Following incubation, the subcultures were visually enumerated. Controls included carrier population control, sterility, purity, neutralization confirmation, and inoculum count.

Note

The Neutralization Confirmation Control performed on August 9, 2012 for SS1691 for both the cotton and polyester carriers did not support the growth of the test organism, *Staphylococcus aureus* did not demonstrate neutralization of SS1684 and SS1685; therefore, test substance neutralization could not be confirmed. All data generated for *Staphylococcus aureus* on August 9, 2012 is considered invalid and is presented in Attachment 1 of the study report.

On September 4, 2012, testing for SS1691 against *Staphylococcus aureus* was repeated using both the cotton and the polyester carriers implementing the filter-neutralization method. The repeat testing performed on September 4, 2012 resulted in a valid Neutralization Confirmation Control. All data generated from September 4, 2012 and all *Enterobacter aerogenes* data for testing performed August 9, 2012 is considered valid and is presented in the body of the report.

No protocol amendments were required for this study. No protocol deviations occurred during this study.

12. **MRID 49081725 "Standard Test Method for Efficacy of Sanitizers Recommended for Soft Non-Food Contact Surfaces (Modification for Spray Product Application)", Test Organisms: *Staphylococcus aureus* (ATCC 6538), for Markie, EPA Reg. No. 3573-RNN, by Anne Stemper, B.S. Study conducted at Accuratus Lab Services. Study completion date – May 17, 2013. Project Identification No. A14755.**

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Three batches (Batch# SS1684, SS1685, and SS1691 [≥60 days old]) of the product, Markie, was tested using Accuratus Lab Services Protocol No. PG30030413.NFS (copy provided). The test substance was received ready to use (RTU). The "initial broth suspension" was prepared by inoculating an initial tube (10mL) of culture broth with 5 transfers from the original stock. A minimum of three daily transfers using 1 loopful (10 µL) of the initial broth suspension into 10mL of culture media was performed on consecutive days prior to use. The appropriate growth medium (Nutrient Broth) was subcultured using a daily transfer (more than 3, but less than 30 transfers) for the organism. A 48-54 hour culture was vortex-mixed and allowed to settle for ≥15 minutes. The upper 2/3rds of the culture was removed and transferred to a sterile vessel for use in testing. The culture was thoroughly mixed prior to use. A 0.10mL aliquot of FBS was added to 1.90mL of each prepared culture to yield a 5% Fetal Bovine Serum organic soil load. Two types of carriers (cotton weave fabric and polyester fabric) were prepared. The cotton weave fabric contained 80 x 80 threads/inch plain cotton weave. A 568.88 gram sample of test fabric was added to 5.7L of scouring solution containing 2.8531 grams of Na_2CO_3, 2.8519 grams of Triton X-100, and 5.7L of deionized water. The polyester fabric was 100% Dacron polyester. A 417.78 gram sample of test fabric was added to 4.2L of scouring solution containing 2.1211 grams of Na_2CO_3, 2.1502 grams of Triton X-100, and 4.2L of deionized water. Each solution was allowed to boil for approximately 60 minutes. The fabric was removed and was rinsed by first placing the fabric into boiling water for a minimum of 5 minutes and then placing the fabric into col water for a minimum of 5 minutes. During the rinsing procedure, the fabric was mixed in order to help remove the wetting agent. The fabric was allowed to air dry. The carriers were cut from the fabric to a size of approximately 1 inch x 1 inch and were autoclave sterilized. After sterilization, each carrier was placed into a sterile Petri dish prior to use in testing. Due to the use of fabric carriers, and the need to meet the minimum control carrier count of 7.5 x 10^5 CFU/carrier, sterile carriers were inoculated with 0.03 mL (30.0 µL) of culture using a calibrated pipettor distributing the inoculum evenly about the carrier. The inoculated carriers were dried for 20 minutes at 35-37°C and 40±2% relative humidity with the Petri dish lids intact. The drying time was reduced to 20 minutes from 35 minutes as stated in ASTM 1153 to increase the likelihood of reaching the minimum control carrier numbers on fabric carriers. The sprayer for each test substance was primed prior to use in testing. After drying, each of the five test carriers for both fabric types was sprayed with the test substance using staggered intervals at a distance of 6-8 inches using 2 sprays and were allowed to expose at room temperature (20°C) and 17% relative humidity for 5 minutes. Following exposure, each carrier was transferred to 20 mL of neutralizer (Letheen Broth + 0.07% Lecithin + 0.5% Tween 80) using identical staggered intervals. Following the neutralization of the test carriers, the excess liquid in each Petri dish was transferred to the neutralizer jar containing the matching carrier. The carriers were vortex-mixed. Within 30 minutes of neutralization, duplicate 1.00mL and 0.10 mL aliquots of the neutralized solution were transferred to individual filter units pre-wetted with 10.0mL of sterile diluent. The contents were evacuated and each filter was rinsed with ≥50mL of sterile diluent. Each filter was transferred to the recovery plate medium (Tryptic Soy Agar with 5% Sheep Blood (BAP)). The plates were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were visually enumerated. Controls included carrier population control, sterility, purity, neutralization confirmation, and inoculum count.

Note

No protocol amendments were required for this study. No protocol deviations occurred during this study.

13. **MRID 49081726 "Standard Test Method for Efficacy of Sanitizers Recommended for Soft Non-Food Contact Surfaces (Modification for Spray Product Application)", Test Organisms: *Escherichia coli* (ATCC 11229), for Markie, EPA Reg. No. 3573-RNN, by Anne Stemper, B.S.**

Study conducted at Accuratus Lab Services. Study completion date – June 29, 2012. Project Identification No. A13412.

This study was conducted against *Escherichia coli* (ATCC 11229). Two batches (Batch# SS1684 and SS1685) of the product, Markie, was tested using Accuratus Lab Services Protocol No. PG30050312.NFS.2 (copy provided). The test substance was received ready to use (RTU). The "initial broth suspension" was prepared by inoculating an initial tube (10mL) of culture broth with 5 transfers from the original stock. A minimum of three daily transfers using 1 loopful (10 µL) of the initial broth suspension into 10mL of culture media was performed on consecutive days prior to use. The appropriate growth medium (Nutrient Broth) was subcultured using a daily transfer (more than 3, but less than 30 transfers) for the organism. A 48-54 hour culture was vortex-mixed and allowed to settle for ≥15 minutes. The upper 2/3rds of the culture was removed and transferred to a sterile vessel for use in testing. The culture was thoroughly mixed prior to use. A 0.10mL aliquot of FBS was added to 1.90mL of each prepared culture to yield a 5% Fetal Bovine Serum organic soil load. Two types of carriers (cotton weave fabric and polyester fabric) were prepared. The cotton weave fabric contained 80 x 80 threads/inch plain cotton weave. A 1206.88 gram sample of test fabric was added to 12L of scouring solution containing 6.0331 grams of Na_2CO_3, 6.1295 grams of Triton X-100, and 12L of deionized water. The polyester fabric was 100 % Dacron polyester. An 80.20 gram sample of test fabric was added to 0.8L of scouring solution containing 0.4032 grams of Na_2CO_3, 0.4199 grams of Triton X-100, and 0.8L of deionized water. Each solution was allowed to boil for approximately 60 minutes. The fabric was removed and was rinsed by first placing the fabric into boiling water for a minimum of 5 minutes and then placing the fabric into col water for a minimum of 5 minutes. During the rinsing procedure, the fabric was mixed in order to help remove the wetting agent. The fabric was allowed to air dry. The carriers were cut from the fabric to a size of approximately 1 inch x 1 inch and were autoclave sterilized. After sterilization, each carrier was placed into a sterile Petri dish prior to use in testing. Sterile carriers were inoculated with 0.02 mL (20.0 µL) of culture using a calibrated pipettor distributing the inoculum evenly about the carrier. The inoculated carriers were dried for 20 minutes at 35-37°C and 40% relative humidity with the Petri dish lids intact. The sprayer was primed prior to use in testing. After drying, each of the five test carriers was sprayed with the test substance using staggered intervals at a distance of 6-8 inches using 2 sprays and were allowed to expose at room temperature (20°C) and 43% relative humidity for 5 minutes. Following exposure, each carrier was transferred to 20 mL of neutralizer (Letheen Broth + 1.0% Lecithin + 9.0% Tween 80) using identical staggered intervals. Following the neutralization of the test carriers, the excess liquid in each Petri dish was transferred to the neutralizer jar containing the matching carrier. The carriers were vortex-mixed. Within 30 minutes of neutralization, duplicate 1.00mL and 0.10 mL aliquots of the neutralized solution (10°) plated onto the recovery plate medium (Tryptic Soy Agar with 5% Sheep Blood (BAP)). The plates were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were visually enumerated. Controls included carrier population control, sterility, purity, neutralization confirmation, and inoculum count.

Note
No protocol amendments were required for this study. No protocol deviations occurred during this study.

14. **MRID 49081726 "Standard Test Method for Efficacy of Sanitizers Recommended for Soft Non-Food Contact Surfaces (Modification for Spray Product Application)", Test Organisms: *Escherichia coli* (ATCC 11229), for Markie, EPA Reg. No. 3573-RNN, by Anne Stemper, B.S. Study conducted at Accuratus Lab Services. Study completion date – June 29, 2012. Project Identification No. A13412.**

This study was conducted against *Escherichia coli* (ATCC 11229). Two batches (Batch# SS1684 and SS1685) of the product, Markie, was tested using Accuratus Lab Services Protocol No. PG30050312.NFS.2 (copy provided). The test substance was received ready to use (RTU). The "initial broth suspension" was prepared by inoculating an initial tube (10mL) of culture broth with 5 transfers from the original stock. A minimum of three daily transfers using 1 loopful (10 µL) of the initial broth suspension into 10mL of culture

media was performed on consecutive days prior to use. The appropriate growth medium (Nutrient Broth) was subcultured using a daily transfer (more than 3, but less than 30 transfers) for the organism. A 48-54 hour culture was vortex-mixed and allowed to settle for ≥15 minutes. The upper 2/3rds of the culture was removed and transferred to a sterile vessel for use in testing. The culture was thoroughly mixed prior to use. A 0.10mL aliquot of FBS was added to 1.90mL of each prepared culture to yield a 5% Fetal Bovine Serum organic soil load. Two types of carriers (cotton weave fabric and polyester fabric) were prepared. The cotton weave fabric contained 80 x 80 threads/inch plain cotton weave. A 1206.88 gram sample of test fabric was added to 12L of scouring solution containing 6.0331 grams of Na_2CO_3, 6.1295 grams of Triton X-100, and 12L of deionized water. The polyester fabric was 100 % Dacron polyester. An 80.20 gram sample of test fabric was added to 0.8L of scouring solution containing 0.4032 grams of Na_2CO_3, 0.4199 grams of Triton X-100, and 0.8L of deionized water. Each solution was allowed to boil for approximately 60 minutes. The fabric was removed and was rinsed by first placing the fabric into boiling water for a minimum of 5 minutes and then placing the fabric into col water for a minimum of 5 minutes. During the rinsing procedure, the fabric was mixed in order to help remove the wetting agent. The fabric was allowed to air dry. The carriers were cut from the fabric to a size of approximately 1 inch x 1 inch and were autoclave sterilized. After sterilization, each carrier was placed into a sterile Petri dish prior to use in testing. Sterile carriers were inoculated with 0.02 mL (20.0 µL) of culture using a calibrated pipettor distributing the inoculum evenly about the carrier. The inoculated carriers were dried for 20 minutes at 35-37°C and 40% relative humidity with the Petri dish lids intact. The sprayer was primed prior to use in testing. After drying, each of the five test carriers was sprayed with the test substance using staggered intervals at a distance of 6-8 inches using 2 sprays and were allowed to expose at room temperature (20°C) and 43% relative humidity for 5 minutes. Following exposure, each carrier was transferred to 20 mL of neutralizer (Letheen Broth + 1.0% Lecithin + 9.0% Tween 80) using identical staggered intervals. Following the neutralization of the test carriers, the excess liquid in each Petri dish was transferred to the neutralizer jar containing the matching carrier. The carriers were vortex-mixed. Within 30 minutes of neutralization, duplicate 1.00mL and 0.10 mL aliquots of the neutralized solution (10°) plated onto the recovery plate medium (Tryptic Soy Agar with 5% Sheep Blood (BAP)). The plates were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were visually enumerated. Controls included carrier population control, sterility, purity, neutralization confirmation, and inoculum count.

Note
No protocol amendments were required for this study. No protocol deviations occurred during this study.

15. **MRID 49081727 "Standard Test Method for Efficacy of Sanitizers Recommended for Soft Non-Food Contact Surfaces (Modification for Spray Product Application)", Test Organisms: *Proteus mirabilis* (ATCC 7002), for Markie, EPA Reg. No. 3573-RNN, by Anne Stemper, B.S. Study conducted at Accuratus Lab Services. Study completion date – June 29, 2012. Project Identification No. A13413.**

This study was conducted against *Proteus mirabilis* (ATCC 7002). Two batches (Batch# SS1684 and SS1685) of the product, Markie, was tested using Accuratus Lab Services Protocol No. PG30050312.NFS.3 (copy provided). The test substance was received ready to use (RTU). The "initial broth suspension" was prepared by inoculating an initial tube (10mL) of culture broth with 5 transfers from the original stock. A minimum of three daily transfers using 1 loopful (10 µL) of the initial broth suspension into 10mL of culture media was performed on consecutive days prior to use. The appropriate growth medium (Nutrient Broth) was subcultured using a daily transfer (more than 3, but less than 30 transfers) for the organism. A 48-54 hour culture was vortex-mixed and allowed to settle for ≥15 minutes. The upper 2/3rds of the culture was removed and transferred to a sterile vessel for use in testing. The culture was thoroughly mixed prior to use. A 0.10mL aliquot of FBS was added to 1.90mL of each prepared culture to yield a 5% Fetal Bovine Serum organic soil load. Two types of carriers (cotton weave fabric and polyester fabric) were prepared. The cotton weave fabric contained 80 x 80 threads/inch plain cotton weave. A 1206.88 gram sample of test fabric was added to 12L of scouring solution containing 6.0331 grams of Na_2CO_3, 6.1295 grams of Triton X-100, and

12L of deionized water. The polyester fabric was 100% Dacron polyester. An 80.20 gram sample of test fabric was added to 0.8L of scouring solution containing 0.4032 grams of Na_2CO_3, 0.4199 grams of Triton X-100, and 0.8L of deionized water. Each solution was allowed to boil for approximately 60 minutes. The fabric was removed and was rinsed by first placing the fabric into boiling water for a minimum of 5 minutes and then placing the fabric into col water for a minimum of 5 minutes. During the rinsing procedure, the fabric was mixed in order to help remove the wetting agent. The fabric was allowed to air dry. The carriers were cut from the fabric to a size of approximately 1 inch x 1 inch and were autoclave sterilized. After sterilization, each carrier was placed into a sterile Petri dish prior to use in testing. Sterile carriers were inoculated with 0.02 mL (20.0 µL) of culture using a calibrated pipettor distributing the inoculum evenly about the carrier. The inoculated carriers were dried for 20 minutes at 35-37°C and 40-41% relative humidity with the Petri dish lids intact. The sprayer was primed prior to use in testing. After drying, each of the five test carriers was sprayed with the test substance using staggered intervals at a distance of 6-8 inches using 2 sprays and were allowed to expose at room temperature (20°C) and 41% relative humidity for 5 minutes. Following exposure, each carrier was transferred to 20 mL of neutralizer (Letheen Broth + 1.0% Lecithin + 9.0% Tween 80) using identical staggered intervals. Following the neutralization of the test carriers, the excess liquid in each Petri dish was transferred to the neutralizer jar containing the matching carrier. The carriers were vortex-mixed. Within 30 minutes of neutralization, duplicate 1.00mL and 0.10 mL aliquots of the neutralized solution (10°) plated onto the recovery plate medium (Tryptic Soy Agar with 5% Sheep Blood (BAP)). The plates were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were visually enumerated. Controls included carrier population control, sterility, purity, neutralization confirmation, and inoculum count.

Note
No protocol amendments were required for this study. No protocol deviations occurred during this study.

V. RESULTS

RTU Spray tested with 5% organic soil load

1. Hard Non-Porous Surface Bactericidal Disinfectant:

Contact Time	MRID No.	Organism	No. Carriers Exhibiting Growth/Total Carriers			Carrier Population (Log_{10} CFU/Carrier)
			Batch #SS1684	Batch #SS1685	Batch #SS1691	
5 minutes	49081714	*Staphylococcus aureus* (ATCC 6538)	0/60	0/60	--	5.74
		Salmonella enterica (ATCC 10708)	0/60	0/60	--	5.79
	49081715	*Staphylococcus aureus* (ATCC 6538)	--	--	0/60	5.91
		Salmonella enterica (ATCC 10708)	--	--	0/60	6.26
	49081716	*Proteus mirabilis* (ATCC 7002)	0/10	0/10	--	6.34
	49081717	*Escherichia coli* (ATCC 11229)	0/10	0/10	--	6.74

2. Hard Non-Porous Surface Virucidal Disinfectant:

Organism	Contact Time	**MRID No.**	**Results**			
				Batch #SS1684	**Batch #SS1685**	**Batch #SS1691**
Influenza A Virus	5 minutes	49081718	**Description**	**Avg. Rep. 1-5**	**Avg. Rep. 1-5**	**Avg. Rep. 1-5**
			10^{-1} to 10^{-7} dilutions	Complete Inactivation	Complete Inactivation	--
			$TCID_{50}$/100µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	--
			Log_{10} Reduction	≥4.68	≥4.68	--
			Average Dried Virus Control ($TCID_{50}$/100µL)	**$10^{5.18}$**		
		49081719	10^{-1} to 10^{-7} dilutions	--	--	Complete Inactivation
			$TCID_{50}$/100µL	--	--	$\leq 10^{0.50}$
			Log_{10} Reduction	--	--	≥4.93
			Average Dried Virus Control ($TCID_{50}$/100µL)	**$10^{5.43}$**		

3. Hard Surface Mildew-Fungistatic:

Contact Time	**MRID No.**	**Organism**	**Visual Evaluation**		**Magnified Evaluation**		***Untreated Carrier Results (Pass/Fail)**
			Batch #SS1684	**Batch #SS1685**	**Batch #SS1684**	**Batch #SS1685**	
Day 7	49081720	*Aspergillus niger* (ATCC 6275)	0% coverage on 10 carriers	0% coverage on 10 carriers	No growth on 10 carriers	No growth on 10 carriers	Pass on 10 carriers

*To be considered passing, each control carrier must demonstrate ≥50% coverage at Day 7.

4. Fabric Mildew-Fungistatic:

MRID No.	**Organism**	**Type of Fabric**	**Contact Time**	**Visual Evaluation**		**Magnified Evaluation**		***Untreated Carrier Results (Pass/Fail)**
				Batch #SS1684	**Batch #SS1685**	**Batch #SS1684**	**Batch #SS1685**	
49081721	*Aspergillus niger* (ATCC 6275) & *Penicillium variabile* (ATCC 32333)	Cotton Muslin Fabric	Day 7	0% coverage on 10 carriers	0% coverage on 10 carriers	No growth on 10 carriers	No growth on 10 carriers	Pass on 10 carriers
			Day 14	0% coverage on 10 carriers	0% coverage on 10 carriers	No growth on 10 carriers	No growth on 10 carriers	Pass on 10 carriers
			Day 21	**5% coverage on 3/10 carriers**	**5% & 10% coverage on 3/10 carriers**	**Growth on 3/10 carriers**	**Growth on 3/10 carriers**	Pass on 10 carriers

		Polyester Fabric	Day 7	0% coverage on 10 carriers	0% coverage on 10 carriers	No growth on 10 carriers	No growth on 10 carriers	Pass on 10 carriers
			Day 14	0% coverage on 10 carriers	0% coverage on 10 carriers	No growth on 10 carriers	No growth on 10 carriers	Pass on 10 carriers
			Day 21	**10% coverage on 1/10 carriers**	**5% coverage on 3/10 carriers**	**Growth on 1/10 carriers**	**Growth on 3/10 carriers**	Pass on 10 carriers

*To be considered passing, each control carrier must demonstrate ≥50% coverage.

5. Hard, Non-Porous, Non-Food Contact Surface Sanitizer:

Contact Time	**MRID No.**	**Organism**	**Results**			**Carrier Population CFU/Carrier (Avg. Log_{10})**
			Batch#	**CFU/Carrier (Average log_{10})**	**Percent Reduction**	
5 minutes	49081722	*Staphylococcus aureus* (ATCC 6538)	SS1684	$<2.00 \times 10^1$ (<1.30)	>99.9	3.02×10^6 (6.48)
			SS1685	$<2.00 \times 10^1$ (<1.30)	>99.9	
			SS1691	$<2.00 \times 10^1$ (<1.30)	>99.9	
		Enterobacter aerogenes (ATCC 13048)	SS1684	$<2.00 \times 10^1$ (<1.30)	>99.9	1.78×10^7 (7.25)
			SS1685	$<2.00 \times 10^1$ (<1.30)	>99.9	
			SS1691	$<2.00 \times 10^1$ (<1.30)	>99.9	

6. Spot Soft, Non-Food Contact Surface Sanitizer:

Contact Time	**MRID No.**	**Organism**	**Results**				
				Plain Cotton Weave		**Polyester**	
			Batch#	**CFU/Carrier (Average log_{10})**	**Percent Reduction**	**CFU/Carrier (Average log_{10})**	**Percent Reduction**
5 minutes	49081723	*Staphylococcus aureus* (ATCC 6538)	SS1684 *[7/6/12]*	$<2.00 \times 10^1$ (<1.30)	>99.9	$<2.00 \times 10^1$ (<1.30)	>99.9
			SS1685 *[7/6/12]*	$<2.00 \times 10^1$ (<1.30)	>99.9	$<2.00 \times 10^1$ (<1.30)	>99.9
			SS1685 *[6/12/12]*	$<2.00 \times 10^1$ (<1.30)	>99.9	--	--
		Carrier Population CFU/Carrier (Avg. Log_{10})		**9.12×10^5 (5.96)** *[6/12/12]*		*[7/6/12]* **1.95×10^6 (6.29)**	
				4.37×10^5 (5.64) *[7/6/12]*			
		Enterobacter aerogenes (ATCC 13048)	SS1684 *[6/12/12]*	$<2.00 \times 10^1$ (<1.30)	>99.9	$<2.00 \times 10^1$ (<1.30)	>99.9
			SS1685 *[6/12/12]*	$<2.00 \times 10^1$ (<1.30)	>99.9	$<2.00 \times 10^1$ (<1.30)	>99.9

		Carrier Population CFU/Carrier (Avg. Log_{10})		*[6/12/12]* **1.23×10^6 (6.09)**		*[6/12/12]* **1.07×10^7 (7.03)**	
	490817 24	*Staphylococcus aureus (ATCC 6538)*	SS1691 *[9/4/12]*	$<2.00 \times 10^1$ (<1.30)	>99.9	$<2.00 \times 10^1$ (<1.30)	>99.9
		Carrier Population CFU/Carrier (Avg. Log_{10}) *[9/4/12]*		**3.63×10^5 (5.56)**		**4.47×10^5 (5.65)**	
		Enterobacter aerogenes (ATCC 13048)	SS1691 *[8/9/12]*	$<2.00 \times 10^1$ (<1.30)	>99.9	$<2.00 \times 10^1$ (<1.30)	>99.9
		Carrier Population CFU/Carrier (Avg. Log_{10}) *[8/9/12]*		**1.48×10^6 (6.17)**		**5.13×10^6 (6.71)**	
	490817 25	*Staphylococcus aureus (ATCC 6538)*	SS1684	$<2.00 \times 10^1$ (<1.30)	>99.9	$<2.00 \times 10^1$ (<1.30)	>99.9
			SS1685	$<2.00 \times 10^1$ (<1.30)	>99.9	$<2.00 \times 10^1$ (<1.30)	>99.9
			SS1691 *[≥60 days old]*	$<2.00 \times 10^1$ (<1.30)	>99.9	$<2.00 \times 10^1$ (<1.30)	>99.9
		Carrier Population CFU/Carrier (Avg. Log_{10})		**1.02×10^6 (6.01)**		**3.39×10^6 (6.53)**	
	490817 26	*Escherichia coli* (ATCC 11229)	SS1684	$<2.00 \times 10^1$ (<1.30)	>99.9	$<2.00 \times 10^1$ (<1.30)	>99.9
			SS1685	$<2.00 \times 10^1$ (<1.30)	>99.9	$<2.00 \times 10^1$ (<1.30)	>99.9
		Carrier Population CFU/Carrier (Avg. Log_{10})		**1.35×10^6 (6.13)**		**1.35×10^7 (7.13)**	
	490817 27	*Proteus mirabilis* (ATCC 7002)	SS1684	$<2.00 \times 10^1$ (<1.30)	>99.9	$<2.00 \times 10^1$ (<1.30)	>99.9
			SS1685	$<2.00 \times 10^1$ (<1.30)	>99.9	$<2.00 \times 10^1$ (<1.30)	>99.9
		Carrier Population CFU/Carrier (Avg. Log_{10})		**1.62×10^6 (6.21)**		**6.61×10^6 (6.82)**	

VI. CONCLUSION

1. The submitted efficacy data **support** the use of the product, Markie, as a broad spectrum disinfectant against the following microorganisms in the presence of 5% organic soil load on hard, non-porous surfaces for a 5-minute contact time:

MRID 490817-14 & -15	*Salmonella enterica* (ATCC 10708), *Staphylococcus aureus* (ATCC 6538)
MRID 490817-16	*Proteus mirabilis* (ATCC 7002)
MRID 490817-17	*Escherichia coli* (ATCC 11229)

Killing was observed in the subcultures of the required number of carriers tested against

the required number of product lots. All tested product lots' active ingredient were at the lower certified limit. Purity controls were reported as pure. Viability controls were positive for growth. Sterility controls did not show growth. Neutralization confirmation testing showed positive growth of the microorganisms.

2. The submitted efficacy data **support** the use of the product, Markie, as a disinfectant with virucidal activity against the following microorganisms in the presence of 5% organic soil load on hard, non-porous surfaces for a 5-minute contact time:

MRID 490817-18 & -19	Influenza A virus, Strain Hong Kong (ATCC VR-544)

Recoverable virus titer of at least 4 $\log_{10}$ were achieved for the required product lots. Complete inactivation (no growth) was demonstrated in all dilutions tested. When cytotoxicity is evident, at least a 3-log reduction in titer was demonstrated beyond the cytotoxic level.

3. The submitted efficacy data **support** the use of the product, Markie, as a hard surface mildew-fungistat against the following microorganisms in the presence of 5% organic soil load on hard, non-porous surfaces for a 7day period:

MRID 49081720	*Aspergillus niger* (ATCC 6275)

These claims are **acceptable** as they are supported by the submitted data.

4. The submitted efficacy data **support** the use of the product, Markie, as a fabric mildew-fungistat on cotton muslin and polyester fabric against the following microorganisms in the presence of 5% organic soil for a 7 and 14 day periods:

MRID 49081721	*Aspergillus niger* (ATCC 6275) *Penicillium variabile* (ATCC 32333)

These claims are **acceptable** as they are supported by the submitted data.

5. The submitted efficacy data **support** the use of the product, Markie, as a ready to use spray non-food contact sanitizer on hard, non-porous surfaces against the following microorganisms in the presence of 5% organic soil load for a 5-minute contact time:

MRID 49081722	*Enterobacter aerogenes* (ATCC 13048), *Staphylococcus aureus* (ATCC 6538)

Results demonstrated a bactericidal reduction of at least 99.9% over the parallel control. Purity controls were reported as pure. Sterility controls did not show growth. Neutralization confirmation testing showed growth.

6. The submitted efficacy data **support** the use of the product, Markie, as a ready to use Spot Soft Surface Sanitizer on soft, inanimate, non-food contact surfaces against the following microorganisms in the presence of 5% organic soil load for a 5-minute contact time:

MRID 490817-23 & -24	*Enterobacter aerogenes* (ATCC 13048), *Staphylococcus aureus* (ATCC 6538)
MRID 49081725	*Staphylococcus aureus* (ATCC 6538)
MRID 49081726	*Escherichia coli* (ATCC 11229)

MRID 49081727 *Proteus mirabilis* (ATCC 7002)

Acceptable killing was observed using the required number of product lots in the subcultures of two types of fabric, cotton and polyester. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth. Neutralization confirmation testing showed growth.

VII. LABEL RECOMMENDATIONS (For label dated 6/10/16)

1. The proposed label claims are acceptable regarding the use of the product, Markie, as a ready to use broad spectrum disinfectant with bactericidal activity against the following organisms for use on hard, non-porous surfaces with spray application for a 5-minute contact time.

   Salmonella enterica (ATCC 10708),
   Staphylococcus aureus (ATCC 6538),
   Proteus mirabilis (ATCC 7002),
   Escherichia coli (ATCC 11229)

   These claims **are supported** by the applicant's data

2. The proposed label claims are acceptable regarding the use of the product, Markie, as a ready to use disinfectant with virucidal activity against the following organisms for use on hard, non-porous surfaces with spray application for a 5-minute contact time.

   Influenza A virus, Strain Hong Kong (ATCC VR-544)

   These claims **are supported** by the applicant's data.

3. The proposed label claims are acceptable regarding the use of the product, Markie, as a hard surface mildew-fungistat against the following organisms for use on hard, non-porous surfaces for a 7day period with spray application.

   Aspergillus niger (ATCC 6275)

   These claims **are supported** by the applicant's data.

4. The proposed label claims are acceptable regarding the use of the product, Markie, as a fabric mildew fungistat against the following organisms for use on cotton and polyester fabrics for a 7 and 14 day contact periods with spray application.

   Aspergillus niger (ATCC 6275),
   Penicillium variabile (ATCC 32333)

   These claims **are supported** by the applicant's data.

5. The proposed label claims are acceptable regarding the use of the product, Markie, as a non-food contact sanitizer against the following organisms for use on hard, non-porous surfaces for a 5-minute contact time with spray application.

   Staphylococcus aureus (ATCC 6538)
   Enterobacter aerogenes (ATCC 13048)

   These claims **are supported** by the applicant's data.

6. The proposed label claims are acceptable regarding the use of the product, Markie, as a Spot Soft Surface Sanitizer against the following organisms for use on soft (fabric) surfaces for a 5-minute contact time with spray application.

   Enterobacter aerogenes (ATCC 13048)
   Staphylococcus aureus (ATCC 6538),
   Proteus mirabilis (ATCC 7002),
   Escherichia coli (ATCC 11229)

   These claims **are supported** by the applicant's data.

7. Additional Label Comments:

   i. **Note to PM**: For Spot Soft Surface Sanitization claims, the label must indicate "For Spot Soft Surface Sanitizing Treatment".

   ii. On page 1 of the proposed label, the directions for use for "mold/mildew prevention" must be revised to "For mold/mildew prevention (control) (inhibition) on soft/fabric surfaces".

   iii. On page 1 of the proposed label, for the directions for use "Sanitization and [Bactericidal & Virucidal] Disinfection", registrant must add "On Hard Non-Porous Surfaces" after "Disinfection".

   iv. On page 1 of the proposed label, registrant must remove the organism, E. aerogenes, from the directions for use for bactericidal and virucidal disinfection (i.e., from the claim "Effective as disinfectant against E. aerogenes, P. mirabilis, E. coli, S. aureus, and Influenza A.") Testing was not done against this microorganism as a disinfectant.

   v. On page 2 of the proposed label, registrant must indicate "Spot Soft Surface Sanitization" in the heading of the section.

   vi. On page 2 of the proposed label, registrant must add "99.9%" to the claim, "Formulated to (remove) (kill) (get rid of) bacteria on (fabrics)…" (i.e., "Formulated to (remove) (kill) (get rid of) 99.9% bacteria…")

   vii. On page 3 of the proposed label, the claim "Prevents bacteria transfers from sheets (pillows) (towels) (mats) (couch) (curtains) (fabrics) (rugs) (blankets) to hands (feet) (face) (fingers)" to "Reduces bacteria transfers from treated surfaces of sheets (pillows) (towels) (mats) (couch) (curtains) (fabrics) (rugs) (blankets) to hands (feet) (face) (fingers".

   viii. On page 3 of the proposed label, registrant must revise the claim "Reduce the spread of bacteria on shared items such as blankets, couches" to "Reduce the spread of bacteria on treated surfaces of shared items such as blankets, couches."

   ix. On page 3 of the proposed label, registrant must remove "kill" from the claim "(This product) is a mildewstat and will effectively (inhibit) (prevent)(kill)(stop) the growth of (mildew) (mold) and the odor caused when (applied) (used) on hard non-porous surfaces for up to 7 days".

   x. Throughout the label, registrant must revise all of the mold/mildew claims of 14 days to specify as contact treatment on soft/fabric surfaces. This means removing all potential hard surfaces, like tile, Jacuzzi surfaces, sinks, tubs, etc. from the claims with a contact period of 14 days and indicating that these claims only pertain to soft/fabric surfaces. Registrant must also remove brackets from "soft surfaces" from claims with a contact period of 14 days. Data submitted only support a 7 day treatment period for hard, non-porous surfaces.

   xi. On page 4 of the proposed label, registrant must remove the brackets from the claim "Eliminates (99.9% of) bacteria, odors and freshens". The log reduction was demonstrated in

the data and must be specified.

xii. Throughout the proposed label, registrant must remove all claims to sanitize and disinfect "more than", "greater than" and "over" 99.9% claims.

xiii. Under section Hard Surface Sanitization Claims on page 4 of the proposed label, registrant must remove "germs" from the claim "Gets to the heart of (the toughest) (tough) odors (by killing 99.9%) (of odor causing germs (bacteria))". This claim only applies to disinfection.

xiv. Also under the section Hard Surface Sanitization Claims, registrant must remove all claims of soft surfaces; these claims may be confusing to the users since the heading title is Hard Surface Sanitization Claims.

xv. On page 4 & 5 of the proposed label, registrant must add "non-porous" to the headings <<Hard surface Sanitization Claim>> and <<Hard Surface Disinfection>>.

xvi. Throughout the label, registrant must revise all "viruses" claims to "virus". Product was only tested against one virus.

xvii. On page 5 of the proposed label, registrant must specify the toilet claim to indicate the exterior of toilets in the claim, "Kills (99.9%) (of) (bacteria) (germs) on (garbage cans) (countertops) (cabinets) (toilets) (non-porous) (hard surfaces)".

xviii. On page 5 & 6 of the proposed label, registrant must remove the following misleading claims:

"Kills bacteria (germs) for as long as you smell the freshness",

"Kills bacteria (germs) (viruses) at the source (root) so they cannot grow (spread)", and

"Kills bacteria (germs) at the source (root) so they cannot grow (spread)".

xix. On page 6 of the proposed label, registrant must specify mold and mildew fungistat in the claim, "Antibacterial (antibacterial action) (antimicrobial) (bactericidal) (bactericide) (fungistat) (spray) (product)".

xx. On page 6 of the proposed label, registrant must remove "(with just 1 spray)" from the claim "Just washed clean and freshness by killing bacteria (germs) and eliminating odors (with just 1 spray)".

xxi. On page 6 of the proposed label, registrant must add "on treated surfaces" to the end of the claim "Reduces risk of cross-contamination from bacteria (germs)".

xxii. On page 6 of the proposed label, registrant must remove the following items under Hard Surface Disinfection:

"(and) (soft surfaces) (surfaces)" from the claim "Kills (99.9% of) bacteria (germs) on (hard surfaces) (and) (soft surfaces)(surfaces) to keep your family (guests) (residents) (customers) (patrons) happy".

"(Enterobacter aerogenes)" from the claim "Kills (eliminates) (reduces) (the following) (household) (away from home) (business)(common) (kitchen) (bathroom) (bacteria) : (Enterobacter aerogenes) (Escherichia coli) (Salmonella enterica) (Staphylococcus aureus) (in 5 minutes) Kills (eliminate) (reduces) in 5 minutes". Disinfection application only pertains to hard, non-porous surfaces and only the microorganisms tested.

xxiii. On page 9 of the proposed label, registrant must remove the claim "Works on a wide range of surfaces". The product is a disinfectant only on hard, non-porous surfaces.

xxiv. On page 9 of the proposed label, registrant must remove the claim "starts working immediately".

xxv. On page 10 of the proposed label, registrant must remove the misleading claim "two sprays a

day gets your through the winter happier".

xxvi. On page 10 of the proposed label, registrant must remove "walk away" from the claim "So easy, you can spray your fabrics & walk away". Users must wait for the contact time before walking away.

xxvii. On page 13 of the proposed label, registrant must remove the surface claim of examination tables, since the claim pertains to medical environment.

xxviii. On page 13 of the proposed label, registrant must specify "toilet seats areas" to be the exterior surfaces of the toilets. Registrant must also remove brackets from "exterior" in the claim "Urinal (exteriors)".